Remarks

Claims 27-30 are currently pending in this application. Applicant has amended claim 27 and 28 herein. Applicant respectfully requests reconsideration of the application.

Applicant has amended claims 27 and 28 to more distinctly claim and specifically point out the inventive aspect of her invention. Reconsideration of the application is requested on that basis.

1. 37 CFR §112/2d Paragraph Rejection

The Examiner has rejected claim 27 under 37 CFR 112/2d for being indefinite. In particular, the Examiner objects to the term "conventional" in the claim in that it is not clear what range of microscopic conditions are claimed.

Applicant would like to point out that the term "conventional" is found in the specification, and generally describes the microscopic conditions. On page 3, lines 25-26 Applicant uses the term to describe a disadvantage of the prior art fluorescent technique: "Additionally, the cost and availability of fluorescent microscopy equipment and trained personnel is greater than *conventional* brightfield microscopy." (Italics added) The term "conventional" is meant to emphasize the following term "brightfield microscopy." "Conventional" is merely an adjective, and is inherent in the term "brightfield microscopy" to the extent that no specialized form of brightfield microscopy is used. And, the specification supports this reading of the term "brightfield microscopy," as it is used throughout the application:

p. 3, lines 31- page 4, line 8:

> "Certain enzymes interact with chromogen substrate solutions to produce distinctly colored products which are capable of being visualized directly through brightfield microscopy. This permits the localization of hybridization sites through enzyme precipitation reactions. Some advantages of cytochemical detection with enzymes include the stability of the precipitate, indicating permanent storage of cell preparations, and the use of a standard brightfield microscope in a setting where routine analysis is performed."

p. 5, lines 28-30:

> "Visualization is typically done by brightfield microscopy (for example in in-situ hybridization), spectrophotometry (for example in solution hybridization) or by visual observation (for example in solid phase hybridization)."

All 3 Figures recite they are brightfield microscopy photographs.

Page 11, lines 14-19:

"The enzyme catalyzes the composition including the color forming compound to provide an insoluble colored composition, which, after optional counterstaining, can be viewed with a brightfield microscope."

Page 17, lines 5-9, outlining on what microscope the Figures were photographed:

"Using brightfield microscopy, microphotographs were made on Kodak Ektachrome color slide film (EL 400), using a Zeiss Axiophot 20 epi-fluorescence microscope (Zeiss, West Germany) equipped with a 100X Plan-Neofluar oil immersion objective or a 100X Plan-APO oil immersion objection (Zeiss, West Germany), a Zeiss MC-100 camera (Zeiss, West Germany), and a blue or neutral density filter."

Clearly conventional brightfield microscopy is the subject of the invention. However, in order to more particularly point out and distinctly claim Applicant's invention and to advance the application, Applicant has amended claim 27 to remove "conventional," because it is inherent in the term "brightfield microscopy" and its inclusion is unwarranted.

2. 35 USC Section 103 Rejection of claims 27-30 over Lizard in view of Bargmann et al. and further in view of MacAulay

Claims 27-30 were rejected under 35 USC 103(a) as being obvious over the combination of Lizard et al. in view of Bargmann et al., US Pat. No. 4935341, and further in view of MacAulay (US 6483641). In particular, the Examiner has stated that Lizard teaches "detecting the chromogenic substrate signal using brightfield microscope conditions." Applicant traverses the rejection for the following reasons.

Lizard et al., the primary reference, teach methods of detecting HPV DNA *in situ* using biotin-labeled DNA probes for either chromogenic (EISH) detection using a brightfield microscope, or fluorescence detection using a laser-scanning confocal microscope (LSCM). The focus of Lizard's teaching is on the enhanced detection of fluorescently-detected probes, versus the chromogenically-detected probes, by use of a LSCM in combination with a CCD camera ("The aim of the present study was to detect by FISH low copy numbers of HPV DNA on whole cells," Id, p. 304, col. 1, lines 20-21). However, Lizard and co-workers compared the chromogenic method (EISH) to the LSCM/CCD camera assisted method. They compared the

results of both methods on Caski, HeLa and SiHa cell lines, which exhibit differing levels of integrated viral DNA. Caski have 600+ copies of HPV DNA type 16, HeLa 10-50 copies of HPV type 18, and SiHa 1-2 copies of HPV type 16. SiHa is of course the most difficult to detect.

In the EISH method, Lizard et al. could not reliably detect HPV in SiHa cells. "With brightfield microscopy and EISH, hybridization spots were observable in Caski and HeLa cells, but hardly any in SiHa cells." Id, col. 1, lines 13-15. In fact, the last sentence of the Abstract states "Single genes of HPV were visualized most efficiently by association of FISH with LSCM or quantitative microscopy with an intensified CCD camera." *Id.*, p. 303, col. 2, lines 5-8. In the Results section, Lizard et al. go into more detail about their EISH results and state that:

> In SiHa cells, hybridization spots were rare and hardly detectable with the HPV 16 probe (Fig. 1D) . . . In SiHa cells, 1-2 spots were sometimes detectable only in a few cells and their size was in the range of those observed in HeLa cells. *Id*, p. 305, col. 2 lines 4-5; lines 14-16.

The Examiner states "Lizard et al teach a method of visually detecting a single copy of the gene in chromosomal DNA in an intact cell using brightfield microscopy." As just discussed, Lizard et al. does not teach single-copy detection of HPV DNA using *in situ* hybridization for brightfield detection. It is clear that Lizard et al. may have seen single copies in rare instances, and failed to see single copies in positive (SiHa) cells. Applicant concludes that Lizard may or may not have been detecting HPV chromogenically in SiHa cell lines-it is not proven in light of Lizard et al.'s own equivocal statements.

Applicant does not claim single copy detection of Her-2/neu using LSCM or other fluorescent technique. Applicant's claim has been, and remains even after amendment, directed to "a method of **visually** detecting a **single copy** of the Her-2/neu gene in chromosomal DNA in an intact cell using **brightfield** microscopy."

Bargmann et al. is cited for teaching a method of detecting the Her-2/neu gene by using a DNA probe to detect point mutations which cause activation of rat neuroblastoma oncogenes. 20-mer probes were provided that were hybridized with rat neuroblastoma lysates subjected to dried gel electrophoresis fractions. (Col. 8, lines 53-60) Bargmann suggests that probes specific to the corresponding human oncogenic point mutations could be found using the same techniques, but do not expressly disclose such point mutations. (Col. 11, lines 39- col. 12, line 13) Bargmann states that "After being radiolabeled, these probes can be used, for example, in

the Southern blot procedure to assess tumor cell DNAs for the occurrence of such point mutations." (Col. 12, lines 4-7) Bargmann does not describe or suggest using such Her2 probes for *in situ* hybridization, as it is well known that to visually detect a gene using chromogenically detectable probes, much longer probes need to be utilized, on the order of at least 50kB. Radiolabeled probes can be exposed to photographic films for long periods of time, thus allowing for single copy detection. However, Applicant's claims are limited to detection by brightfield microscopy, thus excluding radiolabeled probes.

The Examiner combines the method of Lizard et al. and the Her-2/neu probes of Bargmann et al. in an attempt to render *prima facie* obvious Applicant's claimed method. As pointed out above, Lizard et al. do not provide an enabling teaching regarding chromogenic detection of single copies of genes. Instead, Lizard teaches that using LSCM and a CCD camera, one may be able to detect single copies of HPV *in situ* in SiHa cells using fluorescent detection techniques. Lizard admits that they could not reproducibly or reliably detect single copies chromogenically using a light microscope unaided by a CCD camera or other non-standard technique. Bargmann et al. suggest that techniques used to arrive at the rat neuroblastoma 20mer probes could be successfully used to design probes to detect human neu oncogenes. In fact, if the radiolabelled 20mers disclosed in Bargmann were combined with Lizard's method of chromogenic detection, the combination would not arrive at Applicant's Her-2 probes because Applicant used Digoxigenin-labeled Her2 probes of about 80 kb total length. Bargmann's 20 mers would be undetectable chromogenically, or even fluorescently.

MacAulay, US 6483641, is cited by the Examiner for teaching conventional brightfield microscopy. This is simply not correct. The entire disclosure of MacAulay is directed to a 3-D confocal microscope that uses computer-controlled spatial light modulators. See col. 15, lines 14-33; col. 16 lines 3-17; col. 23 lines 53-67. It is a highly complex and expensive system not found in most labs where simple compound light microscopes are used to perform brightfield microscopy. The Examiner's citation to the Abstract, the Claims, and col. 22, lines 58 through col. 23, line 12 is mystifying. They do not support the contention that the patent describes conventional brightfield light microscopy conditions. Therefore, the combination of Lizard, Bergmann and MacAulay does not meet the *prima facie* obviousness standard. Applicant respectfully requests reconsideration on that basis.

Applicant respectfully requests reconsideration of the application on the basis of the above arguments and amendments, and believes the application is now in allowable condition.

Respectfully submitted,

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